

WHAT IS CLAIMED IS:

1. A method of raising and maintaining hematocrit in a mammal comprising administering a therapeutically effective amount of a hyperglycosylated analog of erythropoietin in a pharmaceutical composition, wherein the analog is administered less frequently than an equivalent molar amount of recombinant human erythropoietin to obtain a comparable target hematocrit.
2. The method of Claim 1 wherein the amount of hyperglycosylated analog of erythropoietin is administered about two times per week.
3. The method of Claim 1 wherein the amount of hyperglycosylated analog of erythropoietin is administered about one time per week.
4. The method of Claim 1 wherein the amount of hyperglycosylated analog of erythropoietin is administered about one time every other week.
5. The method of Claim 1 wherein the amount of hyperglycosylated analog of erythropoietin is administered about one time per month.
6. The method of Claim 3 wherein the amount of hyperglycosylated analog of erythropoietin that is administered is about 0.075 to 4.5 μg per erythropoietin peptide per kg per dose.
7. A method of raising and maintaining hematocrit in a mammal comprising administering a therapeutically effective amount of a hyperglycosylated

analog of erythropoietin in a pharmaceutical composition, wherein the analog is administered at a lower molar amount than recombinant human erythropoietin to obtain a comparable target
5 hematocrit.

8. The method of Claim 7 whereas the amount of hyperglycosylated analog of erythropoietin that is administered is about 0.025 to 1.5 µg erythropoietin
10 peptide per kg per dose three times per week.

9. The method of Claims 1 or 7 wherein the hyperglycosylated analog of erythropoietin comprises at least one additional glycosylation site compared to
15 human erythropoietin wherein a carbohydrate chain is added to the site.

10. The method of Claims 1 or 7 wherein the target hematocrit is at least about 30%.
20

11. The analog of Claim 9 wherein the carbohydrate chain is an N-linked carbohydrate chain at one or more of positions 30, 51, 57, 69, 88, 89, 136 and 138 of the sequence of human erythropoietin.
25

12. The analog of Claim 9 which has additional N-linked carbohydrate chains at position 30 and 88 of the sequence of human erythropoietin.

13. The analog of Claim 12 which is
30 Asn³⁰Thr³²Val⁸⁷Asn⁸⁸Thr⁹⁰Epo.

14. The method of Claims 1 or 7 wherein the pharmaceutical composition comprises a pharmaceutically

acceptable diluent, carrier, solubilizer, emulsifier, preservative, and/or adjuvant.

5 15. The composition of Claim 14 wherein the diluent is a buffer solution of sodium citrate or sodium phosphate.

10 16. The composition of Claim 14 wherein the carrier is human serum albumin.

15 17. The composition of Claim 14 wherein the preservative is benzyl alcohol.

20 18. The method of Claims 1 or 7 wherein the mammal suffers from anemia associated with a decline or loss of kidney function.

25 19. The method of Claims 1 or 7 wherein the mammal suffers from anemia associated with myelosuppressive therapy.

30 20. The method of Claim 19 wherein the myelosuppressive therapy comprises chemotherapeutic or anti-viral drugs.

35 21. The method of Claims 1 or 7 wherein the mammal suffers from anemia associated with excessive blood loss.

22. The method of Claims 1 or 7 further comprising administering a therapeutically effective amount of iron.

23. An analog of human erythropoietin comprising at least one additional glycosylation site at any of positions 52, 53, 55, 86 and 114 of the

sequence of human erythropoietin, wherein an N-linked carbohydrate chain is added to the site.

24. The analog of Claim 22 comprising at least two additional glycosylation sites wherein a carbohydrate chain is attached to each of the sites.

25. The analog of Claim 22 comprising at least three additional glycosylation sites wherein a carbohydrate chain is attached to each of the sites.

26. The analog of Claim 22 comprising at least four additional glycosylation sites wherein a carbohydrate chain is attached to each of the sites.

27. An analog of human erythropoietin selected from the group consisting of:

Asn⁵² Thr⁵⁴ Epo;

Asn⁵³ Thr⁵⁵ Epo;

Asn³⁰ Thr³² Val⁸⁷ Asn⁸⁸ Thr⁹⁰ Thr¹²⁵ Epo;

Asn¹¹⁴ Thr¹¹⁶ Epo;

Asn³⁰ Thr³² Asn⁵³ Thr⁵⁵ Val⁸⁷ Asn⁸⁸ Thr⁹⁰ Epo;

Asn⁵⁵ Thr⁵⁷ Epo;

Asn⁸⁶ Val⁸⁷ Thr⁸⁸ Epo;

Ala⁸⁷ Asn⁸⁸ Thr⁹⁰ Epo;

Val⁸⁷ Asn⁸⁸ Ser⁹⁰ Epo;

Val⁸⁷ Asn⁸⁸ Gly⁸⁹ Thr⁹⁰ Epo;

Asn³⁰ Thr³² Asn⁵³ Thr⁵⁵ Epo;

Asn³⁰ Thr³² Asn¹¹⁴ Thr¹¹⁶ Epo; and

Asn³⁰ Thr³² Asn⁵³ Thr⁵⁵ Val⁸⁷ Asn⁸⁸ Thr⁹⁰ Asn¹¹⁴ Thr¹¹⁶ Epo.

28. The analog of Claims 23-27 which is the product of expression of an exogenous DNA sequence.

29. A DNA sequence encoding the analog of Claims 23-27.

30. A eucaryotic host cell transfected with the DNA sequence of Claim 29 in a manner allowing the host cell to express the analog.

31. A composition comprising a therapeutically effective amount of the analog of Claims 23-27 together with a pharmaceutically acceptable diluent, adjuvant or carrier.

32. A method of raising and maintaining hematocrit in a mammal comprising administering a therapeutically effective amount of the analog of Claims 23-27 in a pharmaceutical composition.

33. The method of Claim 32 wherein the analog is administered less frequently than an equivalent molar amount of recombinant human erythropoietin to obtain a comparable target hematocrit.

34. The method of Claim 33 wherein the amount of analog is administered about one time per week, about one time every other week, or about one time per month.

35. The method of Claim 32 wherein the analog is administered at a lower molar amount than recombinant human erythropoietin to obtain a comparable target hematocrit.

36. The method of Claim 34 wherein the amount of analog that is administered is about 0.025 to

1.5 µg erythropoietin peptide per kg per dose three times per week.

37. The method of Claim 34 wherein the amount of analog that is administered is less than about 0.025 µg erythropoietin peptide per kg per dose three times per week.

38. A fusion protein comprising a hyperglycosylated analog of erythropoietin and an immunoglobulin heavy chain constant region.

39. The fusion protein of claim 38 wherein the immunoglobulin heavy chain constant region is an Fc region.

40. The fusion protein of Claim 39 wherein the Fc region is from human IgG or derived from human IgG.

41. The fusion protein of Claim 40 wherein the IgG is IgG1, IgG2, IgG3 or IgG4, or a combination thereof.

42. The fusion protein of Claim 38 comprising at least one additional glycosylation site at any of positions 30, 51, 52, 53, 55, 57, 69, 86, 88, 89, 114, 136 and 138 of the sequence of human erythropoietin, wherein an N-linked carbohydrate chain is added at the site.

43. The fusion protein of Claim 38 which is Asn³⁰ Thr³² Val⁸⁷ Asn⁸⁸ Thr⁹⁰ Epo fused to an Fc region.

